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13. ABSTRACT ***(Maximum 200 Words)***

In order to develop a better understanding of the molecular events that transform normal human prostate cells into prostate cancer, we have developed a system of cell transformation that permits the creation of immortalized and tumorigenic human prostate epithelial cell lines of defined genetic constitution. Expression of SV40 Large T antigen and hTERT, the catalytic subunit of telomerase, permitted immortalization. Transformation as assessed by the ability of these cells to form colonies in an anchorage independent fashion and to form tumors in immunodeficient host animals required the additional expression of an oncogenic version of the H-Ras protein. In addition, using hTERT alone, we have simultaneously created an immortalized human prostate stromal cell line. These cell lines provide an important foundation for future studies that will allow us to investigate the precise molecular interactions that lead to the development of prostate cancer. Ultimately, the elucidation of these critical molecular determinants of prostate cancer will permit the identification and confirmation of important targets for future therapeutic intervention.

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INTRODUCTION

Development of immortalized and tumorigenic prostate cell lines of defined genetic constitution

The study of prostate cancer cells derived from patients has elucidated many fundamental principles of malignant transformation and permitted the identification of several promising therapeutic targets. However, the critical changes that initiate cancer have been difficult to define with this approach. Currently efforts to understand the pathogenesis of prostate cancer are often limited by the difficulty in maintaining and propagating normal and malignant prostate epithelial cells (PrEC) *ex-vivo* as well as by disease and genetic heterogeneity. Specifically, the study of cancer cells derived from patients does not permit one to study the role of particular genes in the stepwise malignant conversion of normal PrEC to cancer cells in a defined genetic background. To address this need, we have developed a system of cell transformation that permits the creation of immortalized and tumorigenic human prostate epithelial cell lines of defined genetic constitution. Using these systems to define these molecular interactions that lead to the final phenotype of cancer will permit us to expand our understanding of prostate cancer and will identify important targets for therapeutic intervention.

BODY

Task 1. Determine which genetic events cooperate with telomerase expression to immortalize prostate epithelial cells

Telomere maintenance by the activation of the reverse transcriptase telomerase leads to immortalization of several types of human cells including fibroblasts, endothelial cells, and retinal pigment epithelial cells (1, 2). However, introduction of the catalytic subunit of telomerase, hTERT, into several types of human epithelial cells, including keratinocytes, airway epithelial cells, and mammary epithelial cells, failed to immortalize these cells despite readily detectable telomerase activity and telomere maintenance (3-5). We found that the introduction of hTERT into primary human PrEC by amphotropic retroviral gene transfer also conferred telomerase activity as measured by the PCR-based telomerase (TRAP, telomere repeat amplification protocol) assay (6) (Fig. 1). As previously noted with normal human fibroblasts, prostate stromal cells are immortalized by expression of hTERT (Fig. 2). In contrast, the expression of telomerase in prostate epithelial cells did not extend the lifespan of such cells (Fig. 2). These observations confirm that PrEC behave similarly to keratinocytes, airway epithelial, and mammary epithelial cells with regard to immortalization by telomerase, specifically that the immortalization of these cells requires the additional introduction of other genetic alterations.

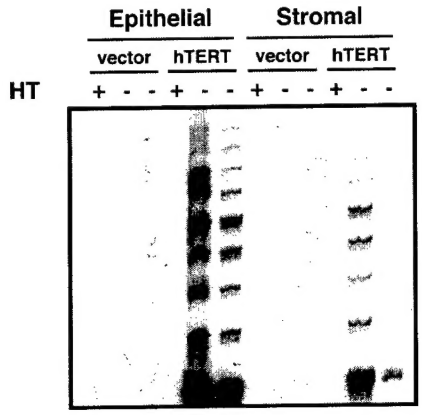

Figure 1. Expression of hTERT in prostate epithelial and stromal cells. hTERT was introduced by retroviral-mediated gene transfer. Telomerase activity determined by the PCR-based TRAP assay. HT refers to heat-treated control.

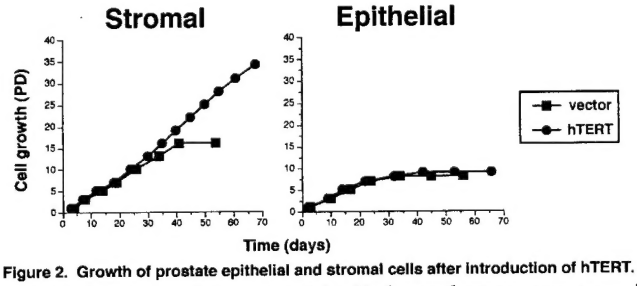

Figure 2. Growth of prostate epithelial and stromal cells after introduction of hTERT.

In ongoing studies in keratinocytes, mammary epithelial, and airway epithelial cells, we have determined that ablation of the retinoblastoma (pRB) and p53 tumor suppressor pathways cooperates with activation of telomerase to immortalize these types of cells (3-5, 7). In order to determine whether PrEC share similar requirements for immortalization, we introduced the SV40 Early Region containing the SV40 large and small T antigens (LT, ST) into PrEC. SV40 LT binds to and inactivates both the pRB and p53 tumor suppressor proteins and together with hTERT expression immortalizes human mammary epithelial and airway epithelial cells (5, 8). After infection, such cells displayed readily detectable expression of LT (Fig. 3). The co-expression of LT and hTERT also permitted

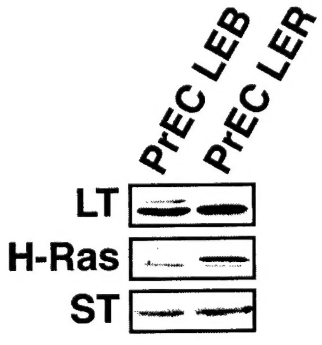

Figure 3. Expression of LT, H-Ras, and ST in PrEC. Antibodies used for detection included Pab101 (LT), C20 (H-Ras), and Pab 419 and Pab 108 (ST).

immortalization of human PrEC (Fig. 4). Immunohistochemical characterization of these immortalized PrEC indicate that they express cytokeratins 5 and 14 and p63 (9) and lack expression of the androgen receptor and PSA, indicating that these PrEC retain a basal phenotype. Thus, we have succeeded in immortalizing both PrEC and prostate stromal cells, a major goal of Task 1.

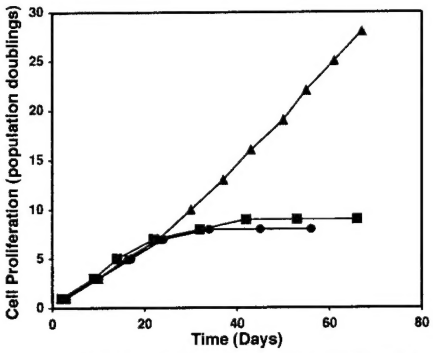

Figure 4. Proliferation of PrEC in vitro. PrEC expressing control vector (circles), hTERT (squares) or LT + hTERT (triangles).

Task 2. Determine which further events are required to convert immortalized cells into tumorigenic cells.

We previously demonstrated that co-expression of SV40 Early Region, hTERT, and *ras* suffices to convert primary human fibroblasts, HEK, mammary epithelial, and small airway epithelial cells to tumorigenicity (5, 8, 10-12). In order to make a genetically defined model of prostate cancer, we wished to determine what further genetic events are required to convert immortalized PrEC into tumorigenic prostate cells. Although the final goal of these experiments is to create an experimental model of prostate cancer that uses genes known to be mutated in human prostate cancer, we have pursued a stepwise strategy to first establish that PrEC are susceptible to this type of *in vitro* transformation assay and then to refine this model through sequential iterations.

Starting with the immortalized PrEC described in Task 1, we introduced an oncogenic allele of the H-*ras* oncogene (V12). Immunoblotting with a pan-Ras antibody revealed that H-Ras was expressed in these cells. We and others have recently determined that in addition to LT, hTERT, and H-Ras, the SV40 ST oncoprotein was required for transformation in human fibroblasts and kidney epithelial cells (7, 12). Since we used the same retroviral vector that was used in these studies in fibroblasts and kidney epithelial cells to introduce the SV40 Early Region into these immortalized PrEC, we expected and confirmed that these cells also expressed ST (Fig. 3). The proliferation rate of these cells did not differ from significantly from the immortalized PrEC when cultured on plastic tissue culture plates (data not shown).

However, when we tested these PrEC expressing LT, ST, hTERT, and H-Ras for their ability to grow in an anchorage-independent fashion, we found that these cells were now able to form colonies in soft agar. Control immortalized PrEC produced in parallel but lacking H-Ras failed to form colonies in soft agar. Correlating with these observations, when we introduced these cells into immunodeficient animal hosts, only PrEC expressing LT, ST, hTERT, and H-Ras were able to form tumors in mice (Table 1). These studies confirm that the additional introduction of H-Ras into immortalized PrEC suffices to confer tumorigenic potential to these cells.

Table 1 Anchorage-independent and tumorigenic growth of PrEC

	Colonies in soft agar	Tumors in animals
PrEC LT + ST + hTERT + vector	-	-
PrEC LT + ST + hTERT + H-Ras	+++	+++

Task 3 Analyze immortalized and tumorigenic prostate epithelial and stromal cells *in vivo* to dissect critical stromal-epithelial interactions

Experiments will commence on this task in the coming year as specified in the original Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS

1. Creation of an immortalized prostate stromal cell line
2. Creation and initial characterization of an immortalized PrEC line
3. Establishment of a system to achieve the transformation of PrEC using sequential gene transfer

REPORTABLE OUTCOMES

1. Development of an immortalized prostate stromal cell line
2. Development of an immortalized PrEC line
3. Development of an immortalized and transformed PrEC line

CONCLUSIONS

In the first year of this award, we have accomplished several of the specific priorities detailed in Statement of Work (Task 1 and Task 2). The creation of these human prostate stromal and epithelial cell lines provide an essential foundation for future studies and represent important reagents for investigators working on prostate epithelial biology. Indeed, these cell lines have already been provided to several other investigators for related but complementary studies.

Although these cell lines represent a significant advance, they are not yet truly representative of spontaneously arising prostate cancer. Furthermore, they retain a basal epithelial cell phenotype. To address these concerns, current and future work will further characterize the biological and phenotypic behavior of these cell lines. In addition, we will now focus on the development of improved prostate cancer cell models that incorporate genetic alterations found associated with spontaneously arising prostate cancer. As detailed in the original proposal and Statement of Work, we will begin by substituting specific dominantly interfering mutants of the pRB and p53 pathways to determine the role of these pathways in immortalization and transformation. In addition, we will begin to develop retroviral libraries derived from prostate cancer cell lines for use in functional screens for genes that are able to substitute for H-Ras expression in this system of transformation. Finally, with the development of an immortalized prostate stromal cell line, we can now begin the experiments outlined in Task 3, which may permit us to generate cell lines that exhibit a luminal phenotype rather than a basal phenotype and which will permit us to study epithelial-stromal interactions during cell transformation.

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APPENDICES

None